Widening of the Excitable Gap and Enlargement of the Core of Reentry During Atrial Fibrillation With a Pure Sodium Channel Blocker in Canine Atria

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Background—This study aimed to assess the effects of pilsicainide, a pure sodium channel blocker, on electrophysiological action and wavefront dynamics during atrial fibrillation (AF).

Methods and Results—In a newly developed model of isolated, perfused, and superfused canine atria (n=12), the right and left endocardia were mapped simultaneously by use of a computerized mapping system. AF was induced with 1 to 5 μmol/L acetylcholine. The antifibrillatory actions of pilsicainide on AF cycle length (AFCL), refractory period (RP), conduction velocity (CV), excitable gap (EG), and the core of the mother rotor were studied. The RP was defined as the shortest coupling interval that could capture the fibrillating atrium. The EG was estimated as the difference between the AFCL and RP. At baseline, multiple wavefronts were observed. After 2.5 μg/mL infusion of pilsicainide, all preparations showed irregular activity, and AF was terminated in 2 preparations. The AFCL and RP were prolonged, and CV was decreased significantly. The EG was widened (147%; P<0.01), and the core perimeter was increased (100%; P<0.01). Increasing the dosage either terminated AF (6 preparations) or converted to organized activity (ie, atypical atrial flutter) (4 preparations). On the maps, all “unorganized” AFs were terminated with the excitation of the core of the mother rotor by an outside wavefront, whereas in preparations with atrial flutter, pilsicainide did not terminate its activity.

Conclusions—Widening of the EG by pilsicainide facilitates the excitation of the core of the mother rotor, leading to the termination of AF. In some experiments, pilsicainide converts AF to persistent atrial flutter. (Circulation. 2003;107:905-910.)

Key Words: atrial fibrillation ■ mapping ■ sodium channel blockers ■ pilsicainide ■ excitable gap

The class IC drug pilsicainide, a pure sodium channel blocker with slow recovery kinetics, has often been used clinically in Japan to terminate paroxysmal atrial fibrillation (AF) and is known for its high conversion rate. Although the mechanism is still unknown, its effects on electrophysiological parameters, such as the refractory period (RP), conduction velocity (CV), and wavelength (WL; the product of RP and CV), have been reported previously. A previous study emphasized prolongation of RP as its antiarrhythmic effect, whereas other studies pointed out the importance of decrement of CV. Recently, it was reported that class I or III antiarrhythmic drugs, such as cibenzoline, quinidine, flecaïnide, and d-sotalol, widened the excitable gap (EG) in a goat model with chronic AF. According to this report, although the effects of these drugs on RP, CV, and WL were diverse, widening of the EG was commonly observed. This study aimed to determine the effects of a pure sodium channel blocker on electrophysiological action and wavefront dynamics during AF.

Methods

Atrial Preparation

A newly developed, isolated, coronary perfused and superfused canine atrial model (Figure 1) was used. This model included the entire right and left atrial walls except the interatrial septum. Twelve healthy dogs of either sex weighing 14 to 18 kg were anesthetized with an intravenous administration of pentobarbital (35 mg/kg IV) and intubated. Under controlled room air ventilation, the heart was rapidly removed through a midsternal thoracotomy and placed in cold, oxygenated Tyrode’s solution. The ventricles were removed, and the atria with the right coronary artery and left circumflex artery were isolated with sharp scissors. To flatten the endocardial side of the tissue, the interatrial septum was carefully removed, and both appendages were opened by cutting along their edges. The specimen was placed endocardial side down in the tissue bath and pinned to the bottom. The tissue size was almost the same as the electrode array. The tissue was perfused immediately at a flow rate of 30 mL/min each via the right and left circumflex coronary arteries and superfused with oxygenated and warm (36.5°C) Tyrode’s solution.
Mapping System and Electrode Array
The CardioMapp System (Prucka Engineering, Inc), the same system as described for a previous ventricle study,7 was used. Data were acquired continuously for 9.7 seconds at a 1-kHz sampling rate with 12-bit accuracy. The signals were obtained from a 4.6×5.4-cm array of 224 bipolar electrodes (14 columns and 16 rows). This array was arranged at the bottom of the tissue bath (Figure 1). The electrodes were connected to the computerized mapping system.

Construction of Isodotted Maps
Activation times were first selected by computer as the time of the fastest slope (dV/dt) of each electrogram. The investigators were then able to select the threshold dV/dt value (a percentage of the dV/dt value). In this study, the threshold values were 20%. The computer selected a time at which the slope of the electrogram exceeded the threshold values. Thereafter, all electrograms were edited manually to correct computer errors. Once the times of activation were determined, these data were entered into a personal computer (PC-MA80T; NEC Inc) to construct isodotted maps. Activation patterns were displayed on the computer screen as colored dots in each 30- to 45-ms consecutive time window. The earliest activation on the isodotted map was colored red, followed by yellow and green. Each color indicates local activation in a settled 10- to 15-ms interval. The red dots represent the electrodes that activated within the last 10 to 15 ms, thus indicating the wavefront.

Induction of AF and Infusion of Pilsicainide
After spontaneous beating of the atria in sinus rhythm had been confirmed, a dosage of 1 to 5 μmol/L acetylcholine (ACh) was added to the circulating and stocked Tyrode’s solution. Then, extrastimuli were applied with a bipolar silver wire. After 8 regular pacings with a twice-diastolic threshold current at a cycle length of 300 ms, premature stimuli were applied. After stimulation had been confirmed for 5 minutes, electrophysiological measurements were started. After acquisition of data for several minutes as control state, a dosage of 2.5 μg/mL of pilsicainide was added to the superfusing and superfusing Tyrode’s solution. After an observation period of 5 minutes, electrophysiological study and data acquisition were performed as the postdrug state. In this study, the cumulative concentration of 7.5 μg/mL was used when AF was not terminated.

Electrophysiological Measurements
Before the present study, 3 preliminary experiments were performed to ensure the stability of electrophysiological measurements, such as AF cycle length (AFCL), RP, CV, and EG, during AF because this experimental model was newly developed. When AF was sustained >5 minutes after the induction in the presence of ACh, these parameters were stable. Therefore, in the present study, data acquisition was started 5 minutes after confirmation of sustained AF.

All of electrophysiological parameters were measured simultaneously in the right and left atria during the steady state of AF. Values were averaged per experiment and then for all experiments.

With our mapping system, we can acquire data for 9.7 seconds at each time. A mean AFCL was measured by an automatic algorithm detecting moments of local activation that were recorded near the site of stimuli placed on the endocardial surface in its period. Definitions of RP, EG, and WL in this study are as follows: RP, the shortest coupling interval that could capture the fibrillating atrium by randomly applied stimuli a total of 200 times (100 times on each free wall of right and left atria); EG, the mean AFCL−RP; and WL=RP×CV. For the measurement of RP, all stimuli were first referred in the local electrograms near the stimulus site to determine the coupling interval. When stimuli were regarded as capture during AF, it was confirmed on the map (Figure 2). The use of a mapping technique to determine RP and EG was also introduced in a previous study.6 CV was measured on the maps. Because the CV of the wavefront is greatly influenced by the curvature,9 a plainly propagating wavefront was taken, and CV was measured in a peripheral lesion of the reentrant wavefronts that appear to be essential in maintaining AF. The period required for 1 complete rotation of the mother rotor was also measured on the maps. At least 10 mother rotors for each preparation were taken before and after administration of the drug.

Measurement of the Core of the Mother Rotor
During AF, the peripheral length of the core of reentry was measured on the computer screen. First, the trajectories of the innermost side of the reentrant wavefront, which appears to be essential in maintaining AF (namely, the mother rotor), were taken. Then, the outermost side of the nonactivated area that located inside of the mother rotor was traced and regarded as the peripheral length of the core. At least 10 mother rotors were taken to estimate it for each episode. Local electrograms with amplitude <10% of the surroundings were silent.

Data Analysis
All statistical analysis was performed with StatView. Results are expressed as mean±SD. A paired t test was used to compare the electrophysiological parameters before and after drug administration.

Results
The experiments, from tissue isolation to data acquisition, took 39±5 minutes (they varied between 32 and 50 minutes) when AF was terminated by pilsicainide.

Wavefront Dynamics During ACh-Induced AF
AF was induced by an extrastimulus method in all preparations, and a concentration of 3.2±0.8 μmol/L ACh was necessary to induce a stable, sustained AF. No episode was induced spontaneously even in the presence of ACh. Irregular and polymorphic activity was seen in each electrogram. Figure 3 shows the wavefront dynamics during ACh-induced
AF. Two to 6 wavefronts were observed on each map, leading to meanderings, breakups, collisions, and the generation of new wavefronts. Although multiple wavefronts were observed on consecutive maps, 1 or 2 mother rotors during AF were found, as shown in Figure 3. In 10 of 12 preparations, a mother rotor occasionally anchored a large pectinate muscle in the right atrium. In such episodes, wavefronts propagated much faster along the pectinate muscle than across it.

Electrophysiological Parameters
For measurements of electrophysiological parameters, including data on the maps, AF episodes from 2 preparations were not available because these 2 AFs were terminated within 5 minutes after infusion (2.5 μg/mL) of pilsicainide and could not undergo subsequent electrophysiological study. The results are summarized in the Table. For the assessment of the postdrug state, all measurements were taken at the same dosage (2.5 μg/mL). Conversion to atrial flutter was not observed at this dosage of the drug. After addition of the drug, the mean AFCL was prolonged ($P<0.001$). From the maps, the period needed for 1 complete rotation of the mother rotor was also prolonged ($P<0.01$). The RP was prolonged ($P<0.01$), and the EG was widened ($P<0.01$). The CV was decreased ($P<0.01$). The measured WL was decreased ($P<0.01$).

Peripheral Length of the Core
Figure 4 shows the wavefront dynamics before and after administration (2.5 μg/mL) of pilsicainide in an episode with the AF unorganized pattern. As shown in this example, the peripheral length of the core of the mother rotor in the postpilsicainide state was prolonged compared with baseline ($P<0.01$).

Activation Patterns After Pilsicainide
At the lower dosage (2.5 μg/mL), all 12 preparations showed unorganized activity. In 4 preparations that AF did not terminate at increased dosage, AF converted to organized activity. In unorganized AFs, although the wavefronts were decreased by pilsicainide, the reentrant wavefronts still ro-

Figure 2. RP and EG were determined by confirming capture and noncapture of randomly applied extrastimuli in local electrograms and on maps. A, Example of capture. During AF with mean AFCL of 127 ms, an extrastimulus with a coupling interval of 85 ms was applied with electrodes from site G13 and was captured in local electrogram. Map shows wavefront propagation in a radial manner from pacing site. B, Example of noncapture. Although a coupling interval of 75 ms was applied from site D2, it was not captured in electrogram or on map.

Figure 3. Isodotted maps and selected bipolar electrograms during ACh-induced AF. A, Note reentrant wavefronts indicated by arrows, which appear to be essential in maintaining AF (ie, mother rotors), although 2 to 6 wavefronts are observed on each map. One or 2 mother rotors are observed in each time window. B, Trajectories of each mother rotor in selected time period. C, Local electrograms obtained from electrode line D from same episode. Period indicated by black arrow at top was analyzed to construct maps.
Effects of Pilsicainide on Electrophysiological Action During AF for 10 Experiments

<table>
<thead>
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<th>Control</th>
<th>Pilsicainide 2.5 μg/mL</th>
<th>P</th>
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<td>81±12</td>
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<td>RP, ms</td>
<td>64±7</td>
<td>90±8 (+41%)</td>
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<tr>
<td>CV, cm/s</td>
<td>75±5</td>
<td>45±5 (-40%)</td>
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<tr>
<td>EG, ms</td>
<td>17±3</td>
<td>42±6 (+147%)</td>
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</tr>
<tr>
<td>Core perimeter, mm</td>
<td>10±4</td>
<td>20±6 (+100%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>WL, cm</td>
<td>4.8±0.4</td>
<td>4.1±0.4 (-15%)</td>
<td>&lt;0.01</td>
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</tbody>
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Pilsicainide widened the EG and enlarged the core of the mother rotor. On an increase in the dosage, AF either terminated or organized into atrial flutter.

Discussion

Our main findings are as follows. Pilsicainide widened the EG and enlarged the core of the mother rotor. On an increase in the dosage, AF either terminated or organized into atrial flutter.

Some previous studies using WL theory have shown the antifibrillatory action of class I and III antiarrhythmic drugs on AF. The importance of WL for the inducibility and stability of AF was initially studied by Rensma et al. and Kirchhof et al. They measured WL during pacing in the absence of AF. As shown in their studies, the antifibrillatory effects of several class I and III antiarrhythmic drugs were related to a prolongation by the minimal WL rather than a more pronounced effect on RP than on CV. Recently, a new technique measuring WL during AF has been introduced by their and other laboratories. Wijffels et al. measured RP with single stimuli applied after every 50 to 100 fibrillation cycles. Starting within the RP, the coupling interval was incremented in steps of 1 to 2 ms. Each stimulus was repeated 10 times; the shortest coupling interval that captured the atrium ≥2 of 10 times was taken as the RP. Capture was verified by activation on the maps. Shinagawa et al. also determined RP by introducing 100 single stimuli after every 6 to 10 signals of atrial activation during AF and took the longest coupling interval that could not capture as the effective RP. In both studies, CV was measured on maps during stable entrainment of AF with an interval equal to the median AFCL. Wijffels et al. concluded that pharmacological cardioversion of AF by class I and III drugs, including the class IC drug flecainide, cannot be explained by a prolongation of WL that was measured during AF, and it may be related to a widening of the EG. The present study measured electrophysiological parameters directly during AF and supports their observations. In contrast, Shinagawa et al. had alternative explanations (see below).

A number of reports have studied the effect of pilsicainide on a vagally induced canine AF model. Although the high conversion effect of this drug was commonly observed in these reports, the effects on RP or CV were diverse. Shinagawa et al. measured such parameters during AF itself and reported that the mechanism of termination is a prolongation of the WL by increasing effective RP without effect on the action potential duration because of prolongation of postpolarization refractoriness with this drug during high-rate AF.

Figure 4. Trajectories of mother rotor before (A) and after (B) perfusion with pilsicainide. Innermost trajectory of reentrant wavefront is indicated by white lines on maps. In this tissue preparation, peripheral length of core of reentrant wavefront is calculated as 10 mm in its prepilsicainide state and 20 mm in its postpilsicainide state. Core peripheral length is prolonged by pilsicainide.
In contrast, 2 studies\textsuperscript{4,5} that measured electrophysiological parameters during pacing have shown alternative results. Hayashi et al\textsuperscript{4} addressed the significance of decrement of CV in termination and the fact that WL was decreased by pilsicainide. Similarly, Iwasa et al\textsuperscript{5} stated the significance of decrement of CV, whereas WL was prolonged with pilsicainide. In the present study, we show electrophysiological data, including EG with wavefront dynamics on maps. Although pilsicainide increased RP during AF, WL shortened because of the high degree of decrement of CV. In addition, AFCL lengthened, and the EG consequently widened. The discrepancy between decreased WL and increased AFCL and core peripheral length can be the result of decreased curvature of reentry. The increased EG is the result of a more marked increase in AFCL than RP.

After pilsicainide, AF either terminated or converted to persistent atrial flutter on an increase in dosage. In a clinical situation, patients who have atrial flutter converted from AF by taking a class IC antiarrhythmic drug are often encountered. Usually, these patients need nonpharmacological cardioversion to eliminate such a drug-induced arrhythmia. The data from this study support this clinical observation. In unorganized AFs, excitation of the central area of the mother rotor by an outside wavefront terminated AF. Before termination, the central area of a mother rotor widened and the period needed for 1 rotation of the reentry was prolonged compared with the control state. Therefore, we believe that the termination occurred because of the presence of a single rotor that maintained AF. Mandapati et al\textsuperscript{13} reported that the size of the core of a stationary spiral wave in an isolated rabbit ventricle was significantly increased by the addition of small concentrations of sodium channel blocker. They suggested that a reduction of sodium current enlarges the core size and prolongs the rotation period, which is consistent with these findings. The idea that reduced excitability makes rotors lose the ability to turn sharply is accepted in the medical community.\textsuperscript{14} Thus, a reduced excitability by sodium channel blockers enlarges the core of the mother rotor and simultaneously widens the EG. It has also been reported that direct excitation of the core terminates a spiral wave of excitation in

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure5}
\caption{Representative episode of an “unorganized” pattern of AF and its termination with pilsicainide. A, Reentrant wavefront meanders from one place to another. Just before termination, an outside wavefront emerges from an excitable area and invades core, leading to termination. B, Selected local electrograms during termination show irregular and polymorphic activity consistent with AF. C, Trajectory of reentrant wavefront is shown on a scheme of preparation.}
\end{figure}

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure6}
\caption{Representative episode of an “organized” pattern AF. A, Single, stationary reentrant wavefront propagated through atria. B, Selected local electrograms show flutter-like activity. C, Trajectory of reentrant wavefront is shown on a scheme of preparation.}
\end{figure}
an atrial model of functional reentry. It may be possible that a widened EG acts against AF just at the termination by allowing outside wavefronts to invade the enlarged core area of reentry through the widened EG area of reentry. These other wavefronts originated from thicker portions of the atrium but not pulmonary veins or ligament of Marshall. Although we could not detect the mechanism of the generation of outside wavefronts that excited the core with our mapping resolution, they may belong to the same wavefront as the originally existing reentrant wavefront, which runs along the epicardium and breaks through to the endocardium. Recently, some role of tissue structure in initiating or maintaining AF, such as pectinate muscle and pulmonary veins, has been reported. In the present study, we found no effects of caval and pulmonary veins. Although some influence of the pectinate muscle on the wavefront behavior was found at baseline, it was decreased by the drug. The effect of the tissue anisotropy may be decreased by pilsicainide. In addition, excitation of the core of reentry occurred in either the right or left atrium. The action of pilsicainide may be independent of tissue structure of the atria.

Study Limitations
First, these data for the healthy heart may not applicable to AF in the presence of organic heart disease. Second, the electrophysiological mechanism of AF may change dramatically after addition of ACh because of the ACh-induced shortening in atrial RP secondary to the effects on the ACh-activated potassium currents, as well as the occurrence of focal atrial activity from which new fibrillatory wavefronts can emerge. Earlier studies have shown focal rapid activity during ACh-induced AF. Therefore, the use of the present animal model may weaken the conclusions in relation to spontaneous atrial fibrillation. Third, the effect of pilsicainide on the nervous system was not investigated because the atria were isolated. Furthermore, wavefront propagation during AF may have been disturbed in the preparation because of the lack of the interatrial septum and the loss of integrity of the atrial appendages. Fourth, although we used bipolar electrograms to construct maps in this study, unipolar electrograms may be more suitable for high-density mapping techniques because the potential difference between the 2 electrodes becomes negligible when both electrode poles activate simultaneously in a small area.

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